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ABNORMAL BARK FORMATION IN *ECHINOPSIS CHILENSIS*, A LONG LIVED TALL COLUMNAR CACTI OF CENTRAL CHILE

FORMACION ANOMALA DE CORTEZA EN ECHINOPSIS CHILENSIS, *UN CACTUS COLUMNAR LONGEVO DE CHILE CENTRAL*

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ABSTRACT

An abnormal epidermal browning has been observed in several long-lived tall columnar cacti in USA, Mexico, Chile, and Argentina; this may promote premature senescence in affected individuals. Although this phenomenon may be associated with important morphological and physiological changes in superficial and internal tissues, anatomical and physiological studies have not been reported yet. Therefore, we determined the anatomical changes that actually occur in the affected surfaces of *Echinopsis chilensis*, a long lived tall columnar cacti from north-central Chile. Results show that epidermal browning is the result of abnormal opaque bark formation originated from the differentiation of cork cambium by epidermic cells. Abnormal formation of overlapping layers of periderm in the surface of the stem results in obstruction of gas exchange and elimination of light access into chlorenchyma. Premature senescence of individuals of this and other tall columnar cacti species in several regions of the world may be the consequence of these malfunctions.

KEYWORDS: *Trichocereus*, bark formation, solar radiation, cacti morphology, global climatic change.

RESUMEN

Se ha detectado en la última década que un gran número de especies de cactus columnares longevos de USA, México, Argentina y Chile desarrollan una coloración café anormal en la epidermis, la que puede causar la muerte prematura de los individuos más afectados. Aunque este fenómeno estaría asociado con cambios importantes en algunas características morfológicas y fisiológicas de los tejidos superficiales e internos del cactus, aún no se han publicado estudios anatómicos ni fisiológicos. En este trabajo determinamos los cambios anatómicos que ocurren en los tejidos superficiales que muestran coloración anómala en *Echinopsis chilensis*, un cactus columnar longevo de la zona norte-central de Chile. Los resultados indicaron que la coloración café es el resultado de la formación inusual de corteza opaca debido a la diferenciación de felógeno a partir de las células epidérmicas. La formación de capas superpuestas de peridermis en la superficie de las columnas produce obstrucción del intercambio de gases y eliminación de la radiación que llega al clorénquima. La muerte prematura de individuos en algunas especies de cactáceas columnares del mundo podría ser explicada por estas malfunciones.

PALABRAS CLAVES: *Trichocereus*, formación de corteza, radiación solar, morfología de cactus, cambio climáuco global.

INTRODUCTION

An increasing mortality of mature saguaro cacti (*Carnegia gigantea* [Engelm.] Britt. & Rose) has been documented in the last decades throughout the Sonoran Desert, USA (Hasting & Turner 1980; Turner 1990; Duriscoe & Graban 1992; Turner 1992). This phenomenon has been coincident with the occurrence of extensive abnormal epidermal browning on the stems of dead and dying cacti (Evans *et al.* 1992; Evans & Fehling 1994; Evans *et al.* 1994a, b). Further studies have shown that abnormal epidermal browning is not restricted to one species or location but it has been occurring in several cacti species in both hemispheres, such as *Pachycereus pringlei*,

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Stenocereus thurberi and *Lophocereus schottii* in the Sonoran Desert, USA, *Trichocereus pascana* and *T. terscheckii* in the North-East of Argentina, and *Echinopsis chilensis* and *E. skottsbergii* in central Chile (Evans *et al.* 1994c; Ginocchio *et al.* 1994; Montenegro *et al.* 1994).

The events involved in epidermal browning follow sheeting and subsequent build up of epicuticular waxes on stem surfaces. This leads to wax sheeting and, eventually, to scaling of stem surfaces with loss of spines as areoles deteriorate, leading to the death of the stem (Evans *et al.* 1992; Evans & Fehling 1994; Evans *et al.* 1994a). Besides the external changes of stem surfaces, the width of internal chlorenchyma is reduced and the colour of the parenchyma changes from pale green in normal cacti to a yellow, yellowish-orange colour, which eventually turns brown in heavily affected cacti (Evans *et al.* 1994a, b). Normal stems of long-lived columnar cacti have tough green surfaces with no flaking or sheeting of any superficial tissue (Mauseth *et al.* 1984), therefore epidermal browning represents an abnormal phenomenon that needs to be further characterized.

In spite of the association of epidermal browning with senescence of tall columnar cacti, the cause of this phenomenon has not been reported yet. At present there seems to be a lack of data suggesting that bacteria, fungi or insects may be causing the shift from a smooth and tough green surface to a wrinkled brown one. Furthermore, current morphological descriptions of changes involved in epidermal browning are not enough to provide a thorough understanding of the changes involved at tissue levels. Therefore, the objective of this work was to determine the anatomical changes which occurred in moderately and heavily affected stem surfaces of *Echinopsis chilensis* (Colla) Friedr. et Rowl., a long-lived tall columnar cacti that dominates the equatorial-facing slopes of the Coastal and Andes Ranges in central Chile (Montenegro 1984).

MATERIALS AND METHODS

Plant material

The same population of *Echinopsis chilensis* (Colla) Friedr. et Rowl. (=*Trichocereus chilensis* B. et R.) described by Evans *et al.* (1994c) as affected by epidermal browning was selected for this study. It is located in a steep equatorial-facing slope near Curacaví (33°24' S, 71°08' W), 30 km North-East of Santiago, Chile. The area has semi-arid Mediterranean climate type, characterized by rainy winters with low temperatures and summer drought periods with high temperatures with an annual precipitation ranging from 200 to 600 mm (Quintanilla 1985).

In November 1991 sixty adult specimens of *Echinopsis chilensis* were chosen for epidermal analysis. Cacti sampled were not selected randomly, rather, an attempt was made to sample cacti with a wide range of surface characteristics in order to analyse tissues with different degrees of epidermal browning. Nomenclature used to define the degree of epidermal browning followed Evans *et al.* (1992). Sampled cacti ranged from healthy green individuals (normal tissue, n=20), light green to brownish-green coloured ones (scaling tissue, n=20), and cacti with thick brown epidermal covering (barking tissue, n=20). Tissue samples of approximately 2x2 cm were taken at a standard height of 1.75 m above ground on the north facing side of the stems from tissues located near the rib crests and rib troughs. The depth of tissue sampling was approximately 1 cm. After tissue samples were removed they were fixed immediately in F.A.A. (formalin-alcohol-acetic acid).

Anatomical analysis

Normal, scaling and barking tissue samples were analysed using optical microscope (OM) and scanning electron microscopy (SEM). Samples were embedded in paraplast, sliced in thin transversal sections (15 mm) and stained with safranin and fast-green for histological analysis at OM (Mauseth *et al.* 1984). SEM samples were dehydrated in an increasingly graded acetone series and dried from 100% acetone via CO_2 in a Polaron E3,000 critical point drying apparatus. Then they were coated with a 100 Å thick golden layer and viewed in the SEM Jeol JSM-25-SII. Each microscope slide and microphotograph was examined at several magnifications to describe and to quantify the process of epidermal browning.

Table 1. Thickness of normal and injuried stem surfaces of *Echinopsis chilensis*.

STEM SURFACE	THICKNESS (µm) * Mean ± S.D.
Normal	374.9 ± 80.00 [a]
Scailing	696.0 ± 83.86 [a]
Barking	1539.0 ± 508.73 [b]

* Different letters indicate significant differences (One way ANOVA and HDS test). Hypodermis thickness was also considered in this measure.

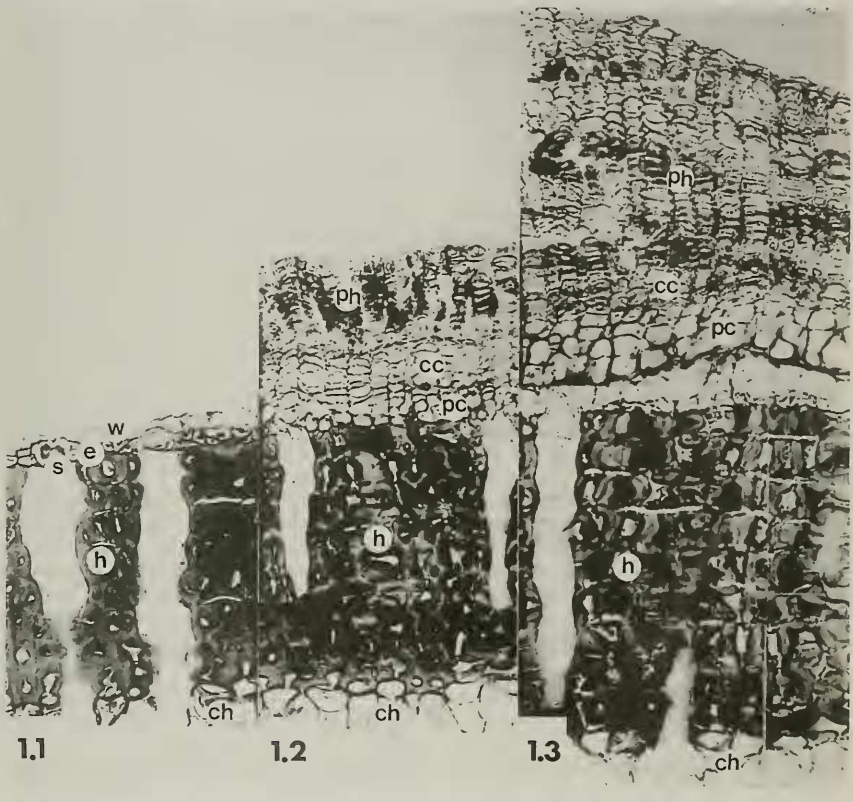

Figure 1.1. Transverse section (x 50) through a normal stem of *Echinopsis chilensis* showing epidermis (e) profusely covered with epicuticular waxes (w), the sunken stomata (s), and the pluristratified hypodermis (h) with very thick walls, interrupted by long sub-stomatic chambers which end in the chlorenchyma (ch). Figure 1.2. Transverse section (x 50) through a scaling stem of *Echinopsis chilensis*, showing periderm developed from a cork cambium (cc) which obstructs stomata. Cell divisions of the cork cambium generate phellem (ph) toward the surface and parenchymatic cells (pc) toward the interior. The hypodermis (h) and chlorenchyma (ch) reamain unaltered. Figure 1.3. Transverse section (x 50) through a barking stem of *Echinopsis chilensis*. A rhytidome structure with several overlaped layers of periderm obstructing stomata is shown by an arrow. Hypodermis (h) and chlorenchyma (ch) reamain unaltered (cc: cork cambium; ph: phellem; pc: parenchymatic cells).

RESULTS

The epidermis of normal stems of *E. chilensis* is composed of a three-celled layer of thin walled small cells covered by a profusion of epicuticular wax produced by the epidermal cells. Below the epidermis, there is a thick hypodermis made of six to eight layers of cells with extremely thick walls (Figures 1.1, 2.1, and 2.2) which leave stomata deeply sunken under the surface. Below the hypodermis there is a parenchymatous cortex composed of several (10-15) layers of columnar palisade chlorenchyma cells and an inner region of non-chlorophyllous parenchyma. Although stomata are located at the surface of the stem, cuticularized stomatal canals run from the stomata through the hypodermis to the assimilatory tissues beneath (Figure 1.1).

Scaling begins at the surface of the stem as small brown dots (Figures 2.3 and 2.4) which expand until they fuse furnishing a bark-like appearance to the whole surface (Figures 2.5 and 2.6). In a transversal section through the dots it is possible to observe formation of phellogen or cork cambium which originates from the reactivation of cellular divisions in the epidermal cells (Figure 1.2). Succesive divisions of the cork cambium result in the formation of radial rows of compactly arranged dead cells toward the surface (phellem), similar to the cork found in woody plants, and in the formation of big spherical parenchymatic cells towards the interior (Figure 1.2). This process results in periderm or bark formation with an associate increase in the stem thickness as it normally occurs during secondary growth of tree trunks. As a result of this process, the external layer of the stem (from hypodermis to the stem surface) is doubled; thus, it ranges from 380 µm in normal surfaces to 696 µm in scaling surfaces (Table I). As figures 1.1 to 1.3 show, the process of bark development is limited to the epidermis and the tissues developed from it, leaving the hypodermis unaltered.

Continuous secondary growth determines the barking appearance of the stem surface (Figures 1.3, 2.5, and 2.6) and a significant increase of the stem thickness (Table I). Additional layers of periderm are differentiated from parenchymatic cells developed from the initially differentiated phellogen. Differentiation of several overlapped layers of periderm leads to a rhytidome-like structure, but this tissue is not a true rhytidome because of the lack of sclerenchymatic fibers as structural components. The outermost periderm layers are shed out as new ones are being formed, resulting in the wrinkled brown appearance of the outer stem surface. Sequential analysis of transversal thin sections of tissue samples indicated that gas-exchange is completely inhibited as overlapping layers of periderm obstruct stomata and lenticels are not formed from phellogen.

DISCUSSION AND CONCLUSIONS

Epidermal browning is not only the result of a build up of epicuticular waxes over the epidermis as it was previously suggested (e.g. Evans *et al.* 1994a). Instead, it results from the replacement of epidermis by periderm as it occurs in secondary growth of tree trunks. Trees typically produce several overlapping periderms (Moore & Clark 1995) but in tall columnar cacti species, where the stem is the main photosynthetic structure, bark tissues transparent to photosynthetic active radiation (PAR) are formed from pure phellem (Mauseth 1988). When stems become older and photosynthesis ceases, a second opaque bark may replace the first one but only at the base of the columns, as it has been described in very old individuals of *Echinopsis chilensis* (Mauseth *et al.* 1984). In most perennial plants, the epidermis and hypodermis are replaced by the periderm (Mauseth 1991). After the formation of the first phellogen in the upper epidermis, parenchymatic cells of the inner cortex may produce another phellogen layer (Mauseth 1991; Moore & Clark 1995). However, in the particular epidermal browning observed in *Echinopsis chilensis,* the epidermis and the cells derived from it originate the periderm, leaving the hypodermis unaltered. Hypodermic cells cannot dedifferentiate in cork cambium because their thick walls constrain normal cell divisions (Mauseth 1988).

Bark formation related to epidermal browning represent an atypical phenomenon in *Echinopsis chilensis* and in other tall columnar cacti because it is not related to edge and it occurs not only at the base of the oldest columns but also very close to the top of the main stems, in photosynthetic

active areas (Evans *et al.* 1992; Evans *et al.* 1994c; Ginocchio *et al.* 1994; Montenegro *et al.* 1994). These opaque periderm layers prevent light reacing chlorenchyma due to their high degree of lignification, thus presumably interrupting photosynthesis and leading to carbon deficit. On the other hand, gas exchange is interrupted due to the lack of appropriate structures in the periderm that connect inner tissues with atmosphere (e.g. stomata, lenticels). Obstruction of gas exchange and screening of light in a high percentage of cacti surface may be a satisfactory explanation for the association of epidermal browning with observed senescence of tall columnar cacti. However, this needs to be demonstrated.

The triggering agent for this abnormal epidermal browning is unknown, but studies of several tall columnar cacti species of both hemispheres have shown significant directional effects, with equatorial stem surfaces showing the strongest epidermal browning (Evans *et al.* 1994a, 1994b, 1994c). These facts suggest that the causal agent is not internal and it is strongly correlated with some environmental factor which follows compass direction. Therefore, it was hypothesized that prolonged exposure to higher solar irradiance could be the causative agent for, or at least contribute to, epidermal browning of long-lived tall columnar cacti (Evans *et al.* 1994a, b, c). Although PAR radiation is about 4 to 5 times greater on surfaces exposed to direct solar irradiance at the latitude where study cacti species grow (calculated from the model of Geller and Nobel 1984), this portion of solar irradiance is harmless to plants and it is necessary for photosynthesis. However, an increase in ultraviolet irradiance on a global scale, resulting from anthropogenic disturbance of the stratospheric ozone layer (Cicerone *et al.* 1974; Grobecker *et al.* 1974; Molina & Rowland 1974) may be the triggering agent of the epidermal browning observed in cacti species located thousands of kilometres away from each other. However, this needs to be demonstrated.

Because UV-B radiation (280-315 nm) is very effective in inducing photochemical reactions in plants (Caldwell 1977, 1981), a slight increase in UV-B irradiance should be deleterious to plants unless they have mechanisms of adaptation against UV-B. Induced biosynthesis of certain UV-absorbing pigments has been described as the most significant avoidance mechanism against enhanced UV-B radiation in foliage (Caldwell 1981). It appears then, that the outer tissues of plant organs may act as UV filters to some extent hence, as a protective mechanism for the plant.

"In cacti species, where the stem constitutes the target tissue for UV light, it has been shown that the epidermis-hypodermis system is, as in many other plants with thick epidermises (Robberech *et al.* 1980), moderately transparent to PAR and thoroughly transparent to near IR. The UV-A and UV-B bands are almost completely absorbed (90%) by the epidermis-hypodermis system (Darling 1989). However, an increased UV-B irradiation on epidermis-hypodermis may lead to changes in stem physiology which induce the formation of a thicker dead cellular layer as a means to reduce transmittance of this radiation to chlorenchymatic tissues and to prevent the excessive loss of water from the stem. This kind of plant response, although effective in restricting the negative effects of UV light on cacti tissues, is ultimately non adaptative. Facultative periderm formation has not been described in other non-woody plants.

In any case, we need to find some evidence that UV radiation has sufficiently increased, both at a local and global basis, to be the causative agent for the opaque bark development observed in tall columnar cacti species. Some indirect evidence of its effects should be provided, based on the frequency of injured stems, and their distribution in various cacti populations across habitats exposed to different levels of ultraviolet solar radiation. If our hypothesis is correct, then the triggering mechanism for this abnormal response should be determined.

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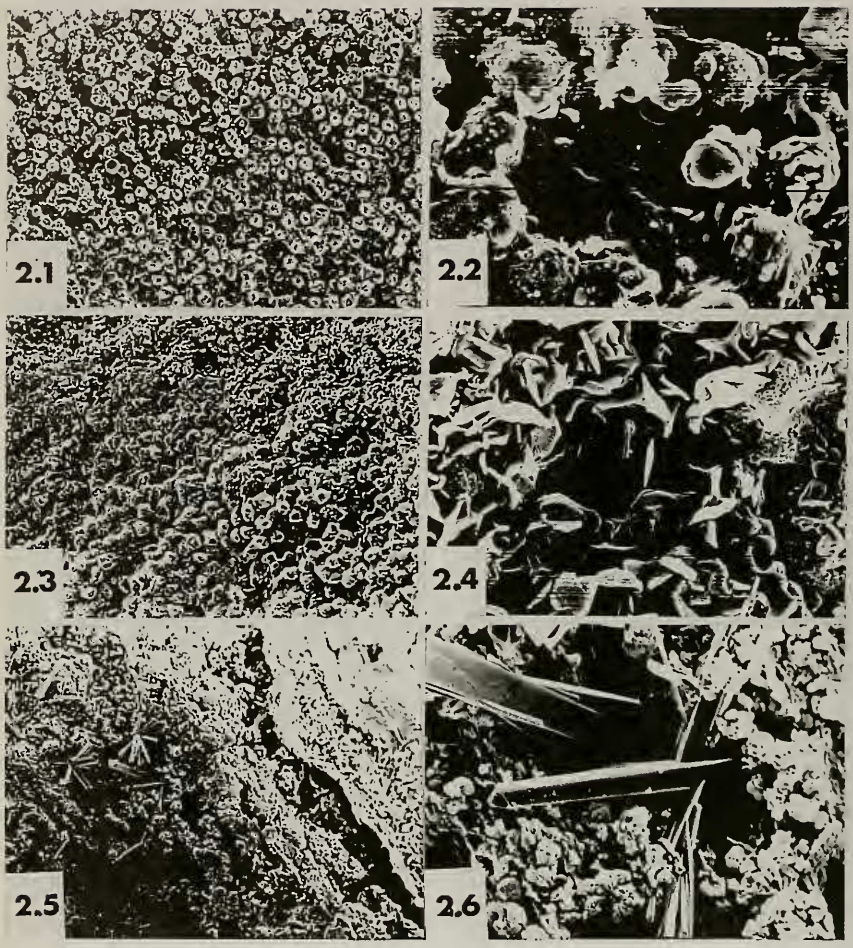

FIGURE 2.1. SEM microphotograph (x 100) of the surface of a normal stem of *Echinopsis chilensis*, profusely covered with epicuticular waxes that have different shapes around stomata. FIGURE 2.2. SEM microphotograph (x 700) of deeply sunken stomata on the surface of a normal stem of *Echinopsis chilensis*. FIGURE 2.3. SEM microphotograph (x 100) of the surface of a scaling stem of *Echinopsis chilensis*. Epicuticular wax is still present but its shape has clearly changed. FIGURE 2.4. SEM microphotograph (x700) of the surface of a scaling stem of *Echinopsis chilensis* that has started the formation of periderm. FIGURE 2.5. SEM microphotograph (x 100) of the surface of a barking stem of *Echinopsis chilensis*. Epicuticular waxes have been completely replaced by a thick opaque bark. FIGURE 2.6. SEM microphotograph (x 700) of the surface of a barking stem of *Echinopsis chilensis*, showing some crystals.

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